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ORIGINAL ARTICLE

Ecological, evolutionary and human-mediated determinants of poeciliid species richness on Caribbean islands

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ABSTRACT

Aim The theory of island biogeography provides a predictive framework relating species richness to island size and distance from the mainland. However, the theory as originally formulated does not necessarily scale to large islands and continental landmasses that are capable of generating species through *in situ* speciation (rather than entirely by colonization), nor does it necessarily account for how human introduction of species alters traditional biogeographical patterns. Here, we examine the ecological (colonization and extinction), evolutionary (*in situ* speciation) and human-mediated (deliberate introductions) determinants of species richness in a taxonomic group that has undergone a radiation on Caribbean islands: live-bearing fishes of the family Poeciliidae.

Location The Caribbean.

Methods We created a database of both native and introduced poeciliid species occurrence on Caribbean islands through literature review, and estimated the number of colonizations versus speciation events on each island using a molecular phylogeny. Linear regression and other statistical tests were used to explore species–area and species–isolation relationships.

Results Species richness on small islands results entirely from colonization and does not significantly increase with island area, whereas on larger islands species richness increases dramatically as a function of area due primarily to *in situ* speciation. Poeciliid fishes have been introduced widely, both as a by-product of their popularity in the aquarium hobby and as a means of mosquito control. We show that such establishments have occurred disproportionately on islands depauperate in native species, and that introduced species richness is positively correlated with economic interconnectedness (shipping traffic) and human population size.

Main conclusions On large Caribbean islands *in situ* speciation has elevated the number of poeciliid species beyond that predicted from ecological processes alone. Introduced species significantly alter biogeographical patterns.

Keywords
biogeography, colonization, dispersal, ecological opportunity, Poeciliidae, radiation, speciation, species–area relationship

INTRODUCTION

According to the theory of island biogeography (MacArthur & Wilson, 1963, 1967), equilibrium species number is determined by two interacting factors: distance from a mainland that serves as the source of colonizing species; and island size, which influences the likelihood of successful establishment (i.e. extinction). This theory has been enormously influential in driving empirical research by allowing both the prediction and interpretation of species–area relationships (Losos &

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Ricklefs, 2010). However, the theory does not readily scale to large islands and continental landmasses because it neglects the macro-evolutionary phenomena of *in situ* speciation, which is more likely to occur on larger islands where the potential exists for niche partitioning and/or allopatric speciation. In a landmark paper, Losos & Schluter (2000) demonstrated a threshold island size above which *in situ* speciation is the predominant factor determining species richness of Caribbean *Anolis* lizards and below which *in situ* speciation did not occur and species richness was governed by colonization and extinction. The idea that *in situ* speciation on larger landmasses outpaces colonization has been explored theoretically (Whittaker *et al.*, 2008; Rosindell & Phillimore, 2011) and demonstrated in other radiations among island archipelagos (Triantis *et al.*, 2008; Kisel & Barraclough, 2010; Losos & Parent, 2010) and lakes (Wagner *et al.*, 2014). In more recent work on Caribbean *Anolis*, Helmus *et al.* (2014) further demonstrated that the establishment of non-native lizard species (owing primarily to unintentional transport during human trade) has strengthened the relationship between island size and species richness while weakening the effect of geographical isolation as a function of distance from the mainland or other sources of colonization.

Islands in the Caribbean region have fostered several other evolutionary radiations, most notably of *Eleutherodactylus* frogs (Hedges, 1989; Heinicke *et al.*, 2007) and fishes in the family Poeciliidae (Rosen & Bailey, 1963; Hamilton, 2001; Doadrio *et al.*, 2009). Poeciliid species have featured prominently in the argument over the merits of vicariance, land bridges and overwater dispersal in explaining current biogeographical patterns (Rivas, 1958, 1986; Rosen & Bailey, 1963; Rosen, 1975; Briggs, 1984; Burgess & Franz, 1989). However, little effort has been devoted to examining the ecological and evolutionary determinants of species richness across the Caribbean. In light of a robust phylogeny for poeciliid fishes (Pollux *et al.*, 2014), we address a number of biogeographical questions using the Caribbean radiation of poeciliid species. Has speciation occurred on any small islands? Does accounting for an evolutionary process (namely *in situ* speciation as a function of island size) improve the explanatory power of the theory of island biogeography? Is there heterogeneity in the species–area relationship among islands of different types (for example those with a continental affinity versus remote oceanic islands)? Is there a negative relationship between distance from a landmass containing potential colonizers and the number of species? Finally, we address how the introduction of non-native poeciliids by humans has modified these relationships, and examine potential predictors of exotic species establishment.

MATERIALS AND METHODS

Database

We created a database of both native and introduced poeciliid species occurrence on Caribbean island banks through literature review (see Appendix S1 in Supporting Information). Island banks encompass individual islands in geographical proximity that share a shallow embankment and were part of a single (larger) island mass when sea levels were lower during the last ice age. We categorized island banks into groupings following Rand (1969) and Losos (1996): Greater Antilles and satellites, Lesser Antilles, isolated islands of the Antilles, and the Bahamas. We added one additional category – 'islands with continental affinity' – which includes those in close proximity to mainland America.

Our database included a number of ecological and habitat variables for each island bank. Island size and maximum elevation were taken from Ricklefs & Lovette (1999), Bass (2003) and Presley & Willig (2008) with some modifications from the CIA World Factbook and WorldBank. Analyses on introduced species included human population size, and a measure of economic isolation taken from Helmus *et al.* (2014), calculated as the total number of ships docking on each bank from anywhere within the Caribbean region in 2007–2008 (Morinière & Réglain, 2012), divided by the maximum value across banks and subtracted from one. We determined each island's geographical isolation using three complementary indices that were informed by minimum area change parsimony ancestral reconstructions for the family Poeciliidae. (1) The shortest distance to the nearest continental landmass (Central or South America) or the shortest distance to Cuba or Hispaniola (since these large islands have served as sources of colonizations for smaller islands). (2) The shortest distance to the nearest continental landmass only (Central or South America). (3) The metric used by Helmus *et al.* (2014) modified based on our current understanding of poeciliid ancestral biogeography. We first took the square root of the potential origins of all Caribbean poeciliid species: total pairwise distance from all banks, distance from Central America, distance from northern South America and total distance from both Central and northern South America. A principal component analysis was performed on these measures and the resulting three orthogonal axes were used as isolation metrics.

Island age has been shown to have an important effect on species diversity on islands (Whittaker *et al.*, 2008; Cameron *et al.*, 2013), and can affect interpretation of species–area and species–isolation relationships (Valente *et al.*, 2014). Data on exactly when each island bank formed as a distinct entity were unavailable, so we used an alternate measure. The Last Glacial Minima occurred in the mid-Pliocene warm period, 3.3–3.0 million years ago. At this time, sea level was 20–30 m above present day sea levels (Dwyer & Chandler, 2009). This would be sufficient to submerge low-lying Caribbean islands and destroy freshwater habitat. Each island was scored for whether or not it would have been submerged during the mid-Pliocene (yes/no).

Poeciliid species on Caribbean islands were compiled from a number of primary sources: Rosen & Bailey (1963), Briggs (1984), Rivas (1986), Rauchenberger (1988), Burgess & Franz (1989), Froese & Pauly (2014) and Eschmeyer & Fricke (2015) as well as several sources for specific islands – Cuba:

Barus & Wohlgemuth (1993); Barus *et al.* (1994); Doadrio *et al.* (2009); Hispaniola: Franz & Burgess (1983); Grand Cayman: Rivas & Fink (1970); Bahamas: Rauchenberger (1989), Smith-Vaniz & Bohlke (1991); Netherlands Antilles: Hulsman *et al.* (2008); Trinidad: Phillip *et al.* (2013). For consistency we used Eschmeyer & Fricke's (2015) Catalog of Fishes as the final authority regarding taxonomic status. Geographical distributions of species on Caribbean islands were taken primarily from Rivas (1958), Lee *et al.* (1983), and Burgess & Franz (1989). Data on introduced species relied heavily on museum collection records found through FishNet2 Portal (www.fishnet2.org). Many islands of the Lesser Antilles have guppies (*Poecilia reticulata*) and/or *P. vivipara*. These species are either known or suspected to be native on islands of the southern Lesser Antilles, closer to their South American mainland source. Perhaps these species naturally extended their range northwards by island hopping, particularly during times of lower sea level. However, several authors (Myers, 1938; Rosen & Bailey, 1963; Briggs, 1984; Burgess & Franz, 1989) have suggested that most of their occurrences on islands in the northern Lesser Antilles (such as St Thomas, Dominica, Barbados and Antigua) are the result of human introductions, and until further (genetic) evidence becomes available we have scored them as such.

Analyses

We primarily used linear regression, with native, introduced, or total species richness as our dependent variable and combinations of island size, geographical isolation, maximum elevation, economic isolation and human population size as our predictor variables. When appropriate, species richness, island size, maximum elevation and human population size were log transformed to improve normality and equality of error variance. Our final data set consisted of all Caribbean island banks that included at least one poeciliid species (either native or introduced). Seven islands had no native species but at least one introduced species. Analyses on native species richness included these islands as zero values. We think this is justified because such islands have suitable habitat to support poeciliids (since introduced species have become established), and the inclusion of these zero-native islands facilitates direct comparison with analyses on total species richness. Statistical analyses were performed in SPSS statistics version 22, graphs were made in SigmaPlot and the isolation metrics developed by Helmus *et al.* (2014) were calculated in R.

The maximum likelihood Poeciliidae phylogeny of Pollux *et al.* (2014) was used to estimate the number of colonizations versus speciation events on each island (see Appendix S2). In addition, we relied on the *Gambusia* phylogenies of Rauchenberger (1989), Lydeard *et al.* (1995) and Langerhans (2011). Within-island speciation is indicated when sister species or larger monophyletic clades occur on the same island (Losos, 1996). We consider it exceedingly unlikely that sister species endemic to a given island each arrived via separate colonization events, with the mainland populations having subsequently gone extinct; the more parsimonious explanation for this pattern is that a single colonizing lineage underwent *in situ* speciation resulting in sister species (or a larger clade) inhabiting a given island.

RESULTS

Five island banks were found to exhibit *in situ* speciation, in decreasing order of size: Cuba, Hispaniola, Jamaica, Great Bank of the Bahamas and Trinidad (Fig. 1). All of these island banks are large (see Appendix S1), the smallest being Trinidad at 4748 km^2, and this island was also recently connected to mainland South America (Kenny, 2008). No cases of *in situ* speciation were observed on smaller islands. Not all species found on Caribbean islands are represented in the poeciliid phylogeny of Pollux *et al.* (2014), leading to uncertainty in the exact number of colonizations and *in situ* speciation events for the islands of Cuba and Hispaniola. Nonetheless, even in these cases the range of estimates can

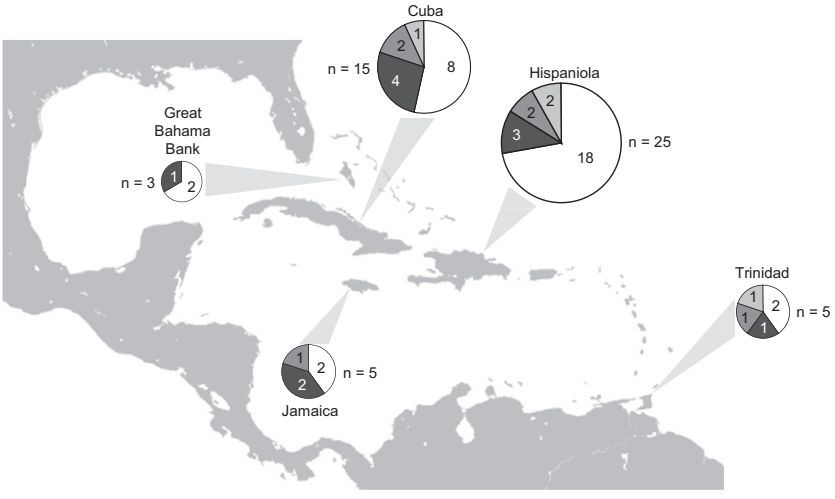

Figure 1 Map of the Caribbean highlighting the five islands where poeciliid species have been generated through both *in situ* speciation and colonization. Pie charts show how native species richness is distributed among lineages (see also Appendix S2). Each lineage present on a given island is represented by a different shade of gray. Hispaniola has the largest radiation; a single colonizing lineage has given rise to 18 species in the subgenus *Limia* (white portion of pie). Relative size of pie chart scales to total number of species present.

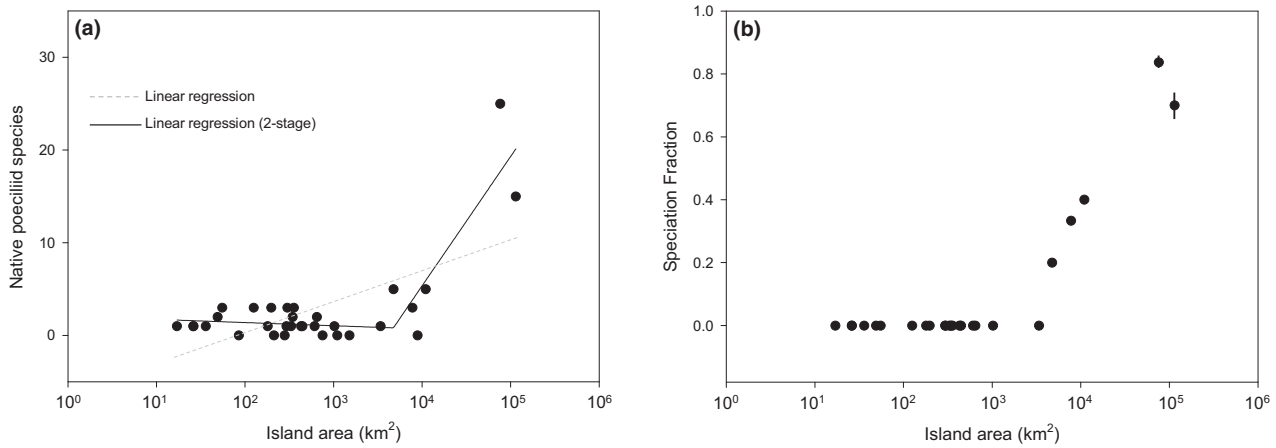

Figure 2 (a) The relationship between Caribbean island size (kilometres squared) and number of native poeciliid species is better fit by a two-stage linear regression (adjusted R^2 = 0.37 vs. 0.78) with optimized break-point at 4728 km^2 ($F_{3,31}$ = 38.1, P < 0.0001). (b) Speciation fraction as a function of island size. The speciation fraction is the number of species generated *in situ* through within-island speciation/the total number of species on a given island including those resulting from both *in situ* speciation and colonizations from elsewhere. The error bars denote the range of estimates resulting from uncertainty in the phylogenetic reconstruction of number of colonizing lineages. The five islands that exhibit *in situ* speciation are in decreasing order of size: Cuba, Hispaniola, Jamaica, Great Bank of the Bahamas and Trinidad.

be narrowed considerably (see Appendix S2). Cuba had 4–5 colonizations and 10–11 *in situ* speciation events giving rise to a total species richness of 15. Hispaniola had 4, 20–21, 25 colonizations, *in situ* events and species richness respectively; Jamaica had 3, 2, 5; the Great Bank of the Bahamas had 2, 1, 3 and Trinidad had 4, 1, 5.

The species–area and species–isolation relationships

Does accounting for evolutionary processes (*in situ* speciation as a function of island size) improve explanatory power for species richness of Caribbean islands? We plotted native species richness as a function of island size to characterize the species–area relationship for Caribbean poeciliid fishes (Fig. 2). This relationship is not well fit by linear regression because the islands of Cuba (15 species) and Hispaniola (25 species) are outliers. Rather, a two-stage regression with an optimized breakpoint at 4728 km^2 provides a good approximation to the data ($F_{3,31}$ = 38.1, P < 0.0001; adjusted R^2 = 0.78). Furthermore, this pattern remains if islands with a continental affinity (many of which were connected to the mainland as recently as the last ice age) were excluded ($F_{3,17}$ = 27.6, P < 0.0001; adjusted R^2 = 0.82). Below this break-point the number of native species does not increase with island area (slope −0.34; P > 0.05); whereas above this threshold there is a significant increase in species number on larger islands (slope 13.94; P < 0.05). This increase is due to the process of *in situ* speciation, present only on larger islands, and driven primarily by the endemic radiations of *Limia* on the island of Hispaniola and *Girardinus* on the island of Cuba (Fig. 2).

Is there heterogeneity in the species–area relationship among islands of different classes? After excluding the Greater Antilles (Cuba, Hispaniola, Jamaica and Puerto Rico) there is no significant increase in native species richness with island size ($F_{1,27}$ = 0.13, P = 0.72; slope = 0.054 ± 0.15 SE). The same result was observed for islands with continental affinity ($F_{1,13}$ = 1.59, P = 0.23; slope 0.20 ± 0.16 SE) and when Lesser Antilles, isolated islands of the Antilles, and the Bahamas were pooled together ($F_{1,13}$ = 0.15, P = 0.71; slope 0.083 ± 0.22 SE). Lastly, we tested for heterogeneity in the species–area relationship among different categories of islands by performing an ANCOVA with island class as categorical variable (again, excluding the Greater Antilles), log island size as covariate and native species richness as the dependent variable. Island type, size and their interaction were all non-significant (P > 0.05). Taken altogether, these analyses indicate a statistically non-significant relationship between island size and species richness on a diverse group of smaller islands.

We used multiple linear regression to test whether a significant relationship exists between the number of native poeciliid species (natural log transformed) and our different indices of geographical isolation, while accounting for the significant positive relationship between island size and species number. We predicted a negative relationship whereby more remote islands contain fewer species. Indeed, we observed a significant relationship regardless of the way in which geographical isolation was calculated (metric 1: $F_{1,31}$ = 5.75, P = 0.023; metric 2: $F_{1,31}$ = 7.02, P = 0.013; metric 3: axis 1, $F_{1,31}$ = 14.15, P < 0.001; axis 2, $F_{1,31}$ = 6.41, P = 0.017; axis 3, $F_{1,31}$ = 0.30, P = 0.589). Furthermore, islands lacking native species are significantly farther from sources of colonization than islands with native species ($F_{1,31}$ = 25.0, P < 0.0001), but there was no significant difference in size between islands with and without native

species ($F_{1,31} = 0.1$, $P = 0.75$). Lastly, we predicted that islands with a continental affinity (that once shared a land connection to a continent during times of lower sea level, and/or remain in close geographical proximity to the mainland) will be closer to species-saturation (equilibrium), as determined by area. In contrast, more remote island banks might be below saturation because of a lack of dispersal events. We first obtained residuals from the species–area relationship (natural log native species richness and log island area). These are an index of how saturated a given bank is, assuming that the saturation curve and a linear species–area relationship have the same slopes; negative residuals indicate impoverishment and positive residuals indicate saturation (Helmus *et al.*, 2014). We found that islands with a continental affinity had significantly more positive 'saturation residuals' than remote islands ($F_{1,31} = 4.16$, $P = 0.05$), suggesting they are closer to saturation.

To assess the original theory of island biogeography we examined species–area and species–isolation relationships using the number of colonizing lineages (Fig. 1). Using colonizing lineages effectively eliminates species generated through *in situ* speciation, and is as if a bank's species richness were entirely determined by colonization and extinction. Here, multiple regression indicates a significant species–area relationship ($F_{1,31} = 7.06$, $P = 0.013$, slope 0.55 ± 0.21 SE) and species-isolation effect ($F_{1,31} = 9.47$, $P = 0.005$, slope -0.003 ± 0.001 SE).

Ecological predictors of native species richness

What factors best explain species richness on Caribbean islands? We used multiple linear regression including island size (log transformed), geographical isolation (metric 1), maximum elevation (log transformed) and island age (submergence during mid-Pliocene) as predictor variables and native species richness (natural log transformed) as the dependent variable. Log island size was a significant predictor of native species richness ($F_{1,31} = 9.9$, $P = 0.004$, slope $= 0.44 \pm 0.14$ SE), as was geographical isolation ($F_{1,31} = 4.7$, $P = 0.039$, slope $= -0.001 \pm 0.001$ SE), but elevation ($F_{1,31} = 0.4$, $P = 0.55$, slope $= 0.16 \pm 0.26$ SE) and island age ($F_{1,31} = 1.2$, $P = 0.29$, slope $= -0.41 \pm 0.38$ SE) were not.

The effects of human-introduced poeciliid species

Introduced species significantly increase species richness across Caribbean islands (paired *t*-test native versus total species, $t = -3.93$, d.f. 31, $P < 0.0001$). We tested several predictions on how the inclusion of these introduced species is expected to alter patterns relating species richness to island size and geographical isolation. First, due to a paucity of competitors, introduced species might be expected to establish on impoverished (species poor) banks. We performed linear regression with 'saturation residuals' (described above) as the independent variable and number of introduced species as the dependent variable. If the slope of this regression is negative then this indicates that introduced species disproportionately establish on impoverished banks. This is indeed what we observe (Fig. 3a; $F_{1,31} = 6.69$, $P = 0.015$; slope -0.68 ± 0.26 SE). The island of Puerto Rico contributes substantially to this phenomenon (Fig. 3b). To illustrate this point, we repeated the analysis but excluded Puerto Rico. Doing so resulted in the regression slope no longer being significant ($F_{1,30} = 1.80$, $P = 0.19$; slope -0.31 ± 0.23 SE). As an alternate measure of bank saturation we used the deviations from a 95% quantile regression of log island size versus natural log native species richness. Results were nearly identical compared to the use of saturation residuals, except that a significant negative relationship remained even after the exclusion of Puerto Rico ($F_{1,30} = 6.3$, $P = 0.018$).

Second, human-mediated colonizations are predicted to diminish the past signal of speciation on bank richness. The inclusion of species introduced by humans lessens the contribution of *in situ* speciation to total species richness, thereby somewhat linearizing the species–area relationship (native species linear regression: $F_{1,31} = 9.6$, $P = 0.004$, adjusted $R^2 = 0.22$; Total species linear regression: $F_{1,31} = 43.4$, $P < 0.0001$, adjusted $R^2 = 0.58$; Fig. 4).

Third, the species–area relationship is predicted to strengthen, while isolation by geographical distance is predicted to weaken, when human-mediated introductions are included in the tallies. We used multiple linear regression with both island size and degree of isolation (distance from nearest mainland or large island) to explain total and native species richness. As expected, the inclusion of introduced species strengthened the species–area relationship (Table 1), while simultaneously weakening the effect of geographical isolation (Table 1).

Lastly, we tested whether economic isolation and population size explain introductions of poeciliid species. If introduction is intentional, then the probability of exotic establishment might be expected to negatively scale with economic isolation (total ships arriving on a given bank) or positively scale with human population size. An island's economic isolation and human population size were each significant predictors of introduced species (Fig. 3), but when multiple linear regression was performed including both variables, only economic isolation remained significant (slope $-3.66 \pm$ SE 1.07, $P = 0.002$).

DISCUSSION

Explanations for patterns of native species richness on Caribbean islands

In both Caribbean *Anolis* lizards and poeciliid fishes we see a similar biogeographical pattern: Below a certain island size, species richness is determined by the ecological processes of colonization and presumably extinction, whereas on larger islands *in situ* speciation contributes substantially to species richness, creating a non-linear scaling pattern with island size

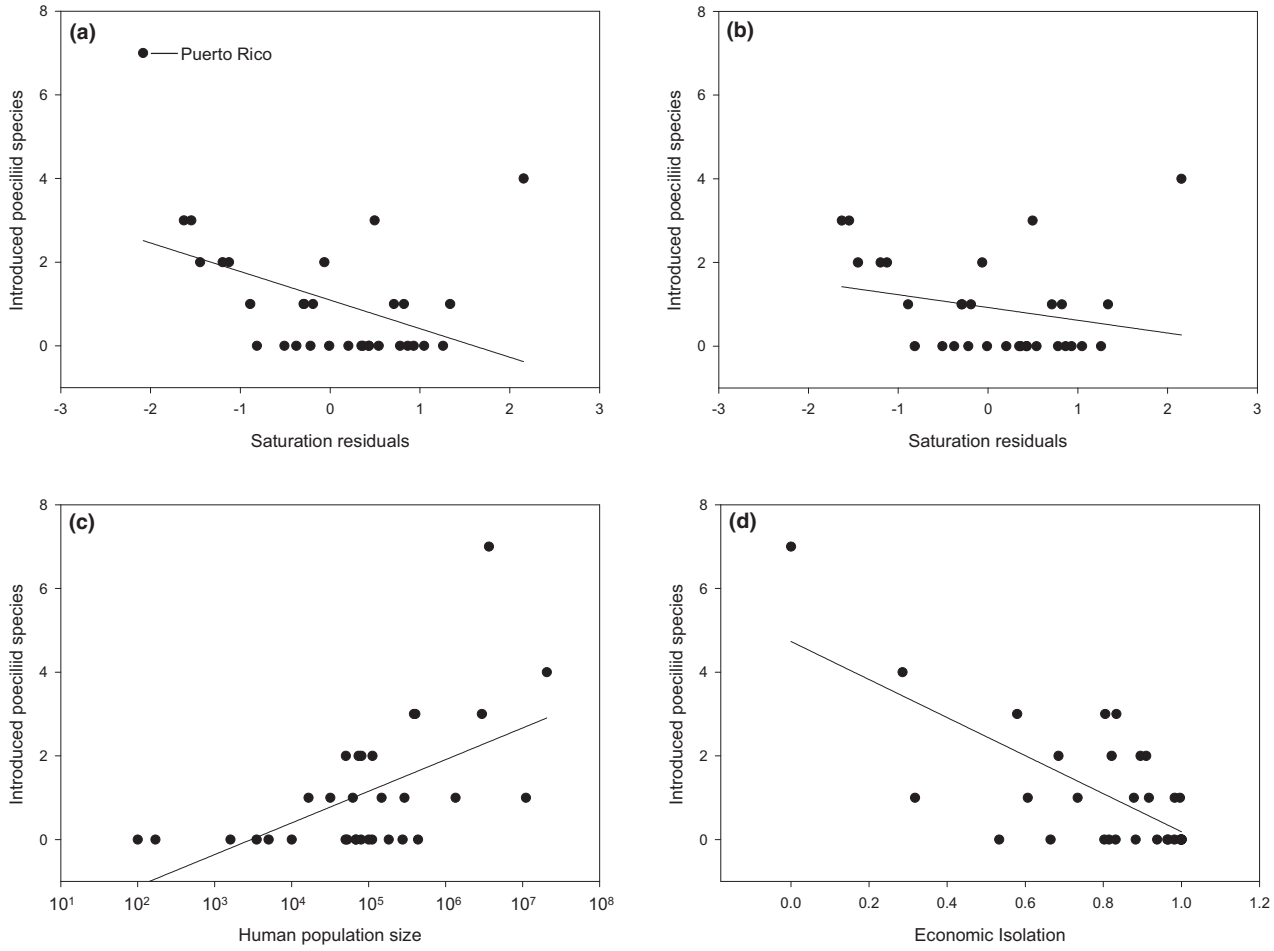

Figure 3 (a) Introduced poeciliid species have become established on impoverished (species poor) banks ($F_{1,31}$ = 6.69, P = 0.015; slope −0.68 ± 0.26 SE). The island of Puerto Rico contributes substantially to this phenomena; Puerto Rico is conspicuous for its large size but complete absence of native freshwater fish species (including poeciliids) with seven introduced poeciliids having become established. (b) When Puerto is excluded the relationship is no longer significant ($F_{1,30}$ = 1.80, P = 0.19; slope −0.31 ± 0.23 SE). (c) The number of introduced poeciliid species shows a significant positive relationship to human population size ($F_{1,31}$ = 14.06, P < 0.001; slope 0.76 ± 0.20 SE). (d) The number of introduced poeciliid species shows a significant negative relationship to an index of economic isolation ($F_{1,31}$ = 28.20, P < 0.001; slope −4.55 ± 0.86 SE).

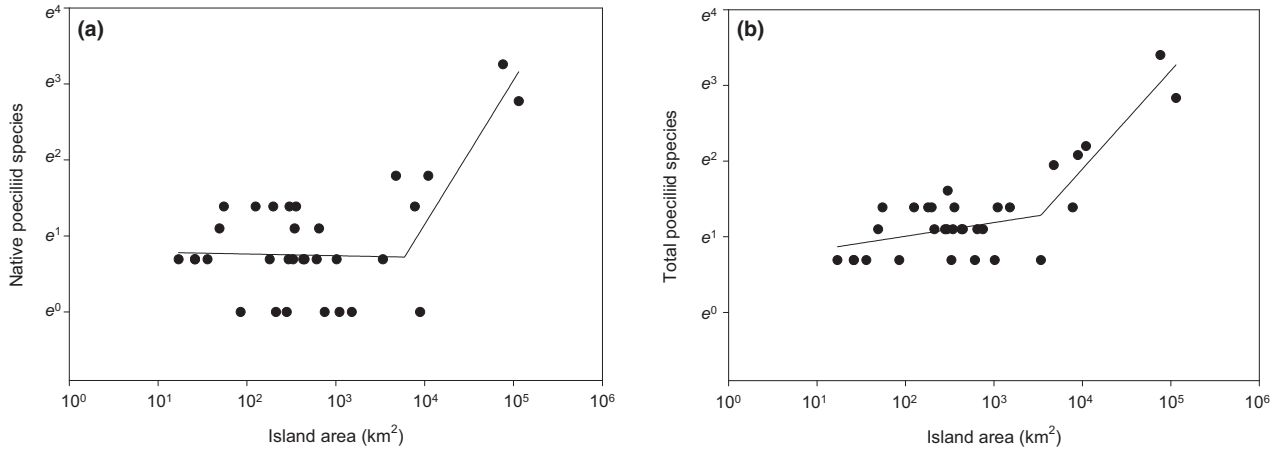

Figure 4 (a) The species–area relationship for native poeciliid species ($F_{3,31}$ = 10.0, P < 0.0001; adjusted R^2 = 0.47) compared to (b) the relationship when species introduced by humans are also included ($F_{3,31}$ = 29.3, P < 0.0001; adjusted R^2 = 0.73). Note scale of y axis is natural log.

Table 1 The importance of Caribbean island size and degree of isolation (distance from nearest mainland or large island) in explaining total and native poeciliid species richness. Total species includes both native and introduced species. As expected the inclusion of introduced species strengthened the species–area relationship, while simultaneously eliminating the effect of geographical isolation. Model was fit using multiple linear regression with explained variation calculated as: (sum of squares of predictor variable/corrected total sum of squares) × 100.

	Sum of squares			Explained variation	
	Island size	Distance	Error (d.f.)	Island size	Distance
Native species	**5.054****	**2.300***	11.602 (29)	27.5	12.5
Total species	**7.403*****	0.047	5.054 (29)	59.4	0.4

***$P<0.001$; **$P<0.01$; *$P<0.05$

that is better accommodated by a two-stage regression. Consideration of these ecological and evolutionary processes allows for a synthesis regarding how Caribbean islands of different sizes have acquired poeciliid species. Small islands have received all their species via colonizations from the mainland or nearby large islands. In several instances, a species has evidently been isolated on a small island and diverged sufficiently from its mainland relative to warrant species status. This fact argues against the notion that small islands lack the requisite habitat for persistence over an evolutionary time-frame. Yet, cladogenetic *in situ* speciation, in which a parent species splits to form two distinct species, is absent on small islands.

Extinction, particularly on small islands, has presumably played a significant role in determining current patterns of poeciliid diversity on Caribbean islands. However, the lack of a fossil record for this group precludes definitive conclusions. Within modern times there have not been historical reports of poeciliid extinctions on Caribbean islands, although information is often scarce. This is particularly true of species in the subgenus *Limia* on the island of Hispaniola, many of which have limited geographical ranges and may be threatened with extinction owing to habitat destruction and predation by introduced species such as tilapia or largemouth bass.

On isolated islands that contain two colonizing *Anolis* species, such species generally differ ecologically, suggesting that for successful establishment a newly colonizing species must be differentiated from the resident (Losos, 1996). On small Caribbean islands that contain several poeciliid species (all of which became established by colonization as opposed to *in situ* speciation), a similar pattern is observed. On Trinidad and Tobago, the closely related *P. reticulata* and *P. picta* exhibit a parapatric distribution (Torres-Dowdall *et al.*, 2013). *Poecilia reticulata's* range extension into lowland rivers seems to be limited by its relative intolerance of brackish and saltwater. *Poecilia picta* is found across a wide range of salinities but its gradual disappearance in more upstream (freshwater) habitat seems to be driven by interspecific competition with *P. reticulata* (Torres-Dowdall *et al.*, 2013). Likewise, on the island of Grenada there are two species of the same genus: *P. reticulata* and *P. vivipara*. *Poecilia reticulata* is found primarily in freshwater and *P. vivipara* is tolerant of saltwater (Burgess & Franz, 1989); furthermore these species also differ in body size. On other small islands in proximity to the American continent (Guanaja, Roatan, Utila, Cozumel, Florida Keys), there is one species of *Poecilia* and one species of *Gambusia*; such species (of different genera) are evidently differentiated enough to permit co-existence.

On mid-sized islands, there is the potential for *in situ* speciation. For example, Jamaica, the Great Bahama Bank and Trinidad have acquired species through a mixture of colonizations from the mainland (or from larger islands) and through limited *in situ* speciation. On each of these islands, sister species exhibit a similar pattern: one species is geographically widespread and the other is found in a single drainage; the species do not occur sympatrically; and they exist in similar habitat and display little morphological differentiation (see Appendix S3) (which has often led to uncertainty regarding taxonomic status and whether they warrant recognition as different species).

Several factors may contribute to large islands supporting extensive *in situ* speciation. Larger island size *per se* may allow greater opportunity for geographical isolation (e.g. in different river drainages or areas separated by a mountain range) and allopatric speciation. A second non-mutually exclusive possibility is that larger islands provide a greater diversity of habitat (ecological opportunity), thereby facilitating niche partitioning and ecological speciation (either in sympatry or allopatry). If allopatric speciation predominates, we would expect monophyletic species groups to be composed of a number of geographically isolated species. In contrast, many species may be found sympatrically and exhibit habitat or ecological specialization. In Caribbean *Anolis*, both of these phenomena apparently contribute to greater species numbers on large islands (Losos, 1996). For example, on Cuba the *sagrei* species group contains 14 ecologically similar species, only two of which have island-wide distributions and most have small geographical ranges. Larger islands (Cuba included) also have sympatric species groups that exhibit habitat subdivision and utilize different ecological niches – termed ecomorphs (Losos, 1996).

On the two largest islands of Cuba and Hispaniola, several poeciliid radiations have followed from a small number of colonization events. Hispaniola has the most endemic species, driven largely by an extensive radiation in the subgenus *Limia* (resulting in 18 recognized species). The greater number of described poeciliid species on Hispaniola is unlikely to be a result of taxonomic artefact or greater ecological opportunity compared to Cuba. Both islands are ecologically complex with high mountains and numerous freshwater habitats.

Hispaniola does have two large brackish lakes – Etang Saumatre and Etang Enriquillo – in the low-lying central cul-de-sac that separates northern and southern portions of the island, but only three *Limia* species are found in this region (Burgess & Franz, 1989). Instead, eight *Limia* species plus one *Gambusia* are found in a much smaller (25 km^2) freshwater lake – Etang de Miragoane – on the southern peninsula (Rivas, 1980; Lee *et al.*, 1983; Burgess & Franz, 1989). Seven of these intralacustrine *Limia* species belong to the same species group (Burgess & Franz, 1989) and presumably constitute a monophyletic clade, thus representing a potential case of within-lake speciation (as is frequently found in cichlids) (Meyer *et al.*, 1990; Schliewen *et al.*, 1994; Barluenga *et al.*, 2006; Seehausen, 2006; Wagner *et al.*, 2014). Little work has been done on the ecology of such species and it remains to be determined whether they exhibit habitat, ecological or trophic specializations – as has been observed in other within-lake radiations (Martin & Wainwright, 2013). Most other endemic *Limia* exhibit limited geographical ranges and are confined to one particular habitat type [such as a sulphur spring (*Limia sulphurophilia*), a single river drainage (*Limia dominicensis*) or mangrove swamps on the nearby Isle de la Gonave (*Limia rivasi*)], with primarily allopatric or parapatric distributions (Rivas, 1980; Lee *et al.*, 1983; Burgess & Franz, 1989). The within-lake radiation of *Limia* on Hispaniola is unique, with nothing comparable having occurred on the island of Cuba. If this one-off event is eliminated, the two islands would have comparable species numbers.

Integrating results from cichlids and poeciliids, it seems that within-lake (perhaps sympatric) speciation boosts diversity at small spatial scales, whereas allopatric speciation acts at much greater spatial scales (also in *Anolis* lizards). There are few natural freshwater lakes on Caribbean islands, which may explain, among other factors, the rarity of within-lake radiations in this group. However, in North, Central and South America there are many freshwater lakes that contain poeciliids that have not undergone within-lake speciation. This may be because in such mainland lakes poeciliids generally have a marginal existence due to an abundance of predators and competitors. In contrast, freshwater lakes on islands often exhibit a reduced ichthyofauna allowing a colonizing species to exploit a greater diversity of lake habitats, which may facilitate speciation.

Introduced species modify biogeographical patterns

The establishment of non-native *Anolis* lizards in a world increasingly interconnected by trade provides novel experimental tests of several biogeographical principles. The primary route of colonization for *Anolis* lizards has been as stowaways on cargo ships transporting ornamental plants between islands or between the mainland and islands (Helmus *et al.*, 2014). The route of establishment for introduced poeciliids is strikingly different. Rather than accidental stowaways, introduction of several common species has likely been intentional, either for mosquito control (*Gambusia affinis*/*holbrooki*) or as a by-product of the aquarium hobby (*P. reticulata*, *Xiphophorus helleri*). Two conditions are required for the successful establishment of poeciliid species: suitable permanent freshwater or brackish habitat, and the deliberate release of founding stock into such habitat. We expected the second requirement might be largely idiosyncratic and, thus (unlike *for Anolis* lizards), unlikely to yield informative biogeographical patterns. Alternatively, we considered the possibility that the likelihood of release of founding stock is positively related to human population size or negatively related to how economically isolated an island is.

Introduced species have become disproportionately established on species-poor islands. This phenomenon is driven by Puerto Rico, which inexplicably contains no native primary freshwater fish but has much suitable habitat (with seven introduced poeciliid species having become established) (Burgess & Franz, 1989). Extinction or an island's lack of colonizing lineages (owing to geographical isolation or a host of other factors) could keep it well below its theoretical saturation limit, as apparently was the case with Puerto Rico. Furthermore, geographically remote small islands that are depauperate in native species can exhibit suitable habitat. In both cases, human introductions have filled these ecological voids.

We also found evidence that introduced species richness is related to human population size and economic interconnectedness. One potential explanation for the significant positive relationship with human population size is that the likelihood of non-native species being released increases as a function of the number of people keeping such fish in home aquaria. The explanation for the relationship between economic isolation and introduced species richness is more tangential. Perhaps, islands that are more economically isolated (i.e. receive less shipping traffic) receive fewer imported poeciliids or have fewer aquarists resulting in reduced likelihood of deliberate introductions. Here again, Puerto Rico presents an interesting case. It has by far the most introduced species (seven) and is also the least economically isolated Caribbean island, likely owing to its status as a U.S. territory.

Continental islands (being near or at times even connected to a mainland source) are predicted to be close to their equilibrium species number as determined by area. That is, for these proximate islands, geographical isolation is predicted to be far from limiting and, thus, island size is the primary determinant of species number. By contrast, the lack of chance dispersal events may keep remote islands well below saturation, although they may have requisite habitat for species establishment. The introduction of species to such islands is thus expected to strengthen the species–area relationship by bringing all islands closer to their area-determined saturation point. The converse is that in the modern age of ship and jet travel, geographical distance does not necessarily determine which islands introduced species are likely to become established, and this is predicted to weaken the effect of isolation-by-distance. In this study, the inclusion of introduced poeciliid species strengthened the species–area relationship, while simultaneously eliminating the isolation-by-distance effect.

The patterns uncovered in this study on Caribbean poeciliid fishes largely mirror those found in *Anolis* lizards in the same biogeographical region (Losos, 1996; Losos & Schluter, 2000; Helmus *et al.*, 2014). Whether these patterns are general and apply to other island archipelagos worldwide or are unique to the Caribbean is an interesting empirical question that awaits future study.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Poeciliid species occurrence on Caribbean island banks obtained through literature review.

Appendix S2 Summary of poeciliid colonizing lineages and speciation events.

Appendix S3 Geographical distribution and evidence for ecological specialization in Caribbean poeciliids.

DATA ACCESSIBILITY

The full data set used in all analyses has been deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.08m53.

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